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BIOSYSTEMATICS OF AEDES (NEOMELANICONION)

Annual Report

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June 1988

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-86-C-6134

University of San Francisco
San Francisco, California 94117-1080

ADA197427

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved
OMB No 0704-0188
Exp Date Jun 30, 1986

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: University of San Francisco Department of Biology; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code): San Francisco, CA 94117-1080	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION: U.S. Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: DAMD17-86-C-6134
8c. ADDRESS (City, State, and ZIP Code): Fort Detrick, Frederick, Maryland 21701-5012	10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO	PROJECT NO	TASK NO	WORK UNIT ACCESSION NO
62770A	3M1 62770A871	AB	384

11. TITLE (Include Security Classification): Biosystematics of *Aedes* (*Neomelaniconion*).

12. PERSONAL AUTHOR(S): Zavortink, Thomas James

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 5/5/87 TO 5/4/88	1988, June	20

16. SUPPLEMENTARY NOTATION

17. COSATI CODES		
FIELD	GROUP	SUB-GROUP
06	03	
06	13	

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number): Taxonomy, Biosystematics, Mosquito Taxonomy, *Aedes*, *Neomelaniconion*, RA 1

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

See Reverse

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☒ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325; 22c. OFFICE SYMBOL: SGRD-RMI-S

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

19. Abstract

The objective of the "Biosystematics of *Aedes (Neomelaniconion)*" project is to produce a modern taxonomic monograph of the aedine subgenus *Neomelaniconion*. Comparative morphological taxonomic procedures will be emphasized. Characteristics from both sexes and all stages of the life cycle will be studied.

During the second contract year, additional facilities and equipment were acquired. Specimens of *Neomelaniconion* were obtained through loans from museums in France and South Africa and through field work by staff and cooperators in Ivory Coast and South Africa. A total of 169 field collections was made. Over 200 egg clutches obtained in the field were hatched in the laboratory and progeny series were reared. Approximately 3800 adult mosquitoes, 1500 immature mosquitoes, and 100 male genitalia were prepared for study. Approximately 75% of this material was permanently labeled. All specimens of *Neomelaniconion* acquired by the project to date have been provisionally identified, and 28 species are represented. A partial or complete set of the preliminary drawings of the larva, pupa, and male genitalia of nine species of *Neomelaniconion* was completed. Taxonomic study has shown that numerous undescribed species of *Neomelaniconion* exist and that the current status of some nominal taxa must be changed. Eggs of *Neomelaniconion* show valuable taxonomic characters. Enzyme analysis of several species of *Neomelaniconion* revealed biochemical differences between the species. Species that belong to the forest group are well-differentiated from each other by larval and male genitalic characters, whereas those in the savanna group are not. Species in the savanna group have a greater number of diagnostic isozymes. Adult *Neomelaniconion* are difficult to identify to species because of variation in their external characters.

Contents

Statement of the Problem

The objective of the project "Biosystematics of Aedes (Neomelaniconion)" is to produce a modern taxonomic monograph of this subgenus of mosquitoes. This subgenus, which is primarily Ethiopian in distribution, has never been the subject of a thorough taxonomic study, and so its species remain poorly known. The absence of basic information on the number of species in the group and of reliable keys for their identification severely hampers the acquisition and reporting of biological information about these mosquitoes. The result is that the distribution, bionomics, and disease vector potential of the different species remain unknown or uncertain.

Species of Neomelaniconion are believed to be involved in both the inter-epizootic maintenance and transmission of Rift Valley fever virus. A complete understanding of the natural history of this virus is not possible without better knowledge of these mosquitoes.

Background

As it is presently understood, the subgenus Neomelaniconion includes 28 nominal species, 24 of which are considered to be valid taxonomic species or subspecies (1-3). All except one of the currently recognized species are restricted to the Ethiopian Region. The exception is Aedes lineatopennis (Ludlow), which is widespread in the Oriental and Australian regions.

The existing taxonomy of the subgenus Neomelaniconion dates back to Edwards's treatment of the group under its former name, Banksinella Theobald, in his catalog of the family Culicidae (4) and in his volume on adult Mosquitoes of the Ethiopian Region (5). Edwards's studies were based almost entirely upon adult mosquitoes, and characteristics of the immature stages were not considered. In the many decades since Edwards's brief taxonomic treatments of Neomelaniconion, there has been no comprehensive study of the group. Several additional species have been described (3, 6-10), immatures of a few species have been partially described or illustrated (7, 9, 11-17), one nominal species has been transferred to the subgenus (18), and two nominal species have been removed (19).

In the absence of a comprehensive study of Neomelaniconion, the subgenus remains poorly and inadequately known. The immature stages, in particular, have been neglected. They have never been used to help define the species of the group or to help place these species into a

natural classification. In fact, to this day the immatures of nearly half the species of Neomelaniconion are unknown, and for those species in which they are known, they have been described and illustrated very superficially. The complete larval and pupal chaetotaxy has not been studied for a single species. Available keys to adults (5, 9, 15) and larvae (11, 15) of Neomelaniconion are inadequate because they treat only a portion of the species now known or treat only the species of a restricted region.

Numerous arboviruses have been isolated from species of Neomelaniconion (20). The virus that causes Rift Valley fever, an important disease of domestic animals and humans in Africa and a potential international disease problem (21), is the most important of these. This virus has been isolated from field populations of three or more species of Neomelaniconion, circumluteolus (Theobald) in South Africa (22) and Uganda (23), lineatopennis in Kenya (24), South Africa (25), and Zimbabwe (26), palpalis (Newstead) in Central African Republic (27), and possibly luteolateralis (Theobald) in South Africa (28). Recently reported laboratory experiments have shown that it can be transmitted horizontally by yet another species of Neomelaniconion, unidentatus McIntosh (29). Studies of Rift Valley fever in Kenya have provided evidence that lineatopennis is a reservoir for the virus between epizootics, transmitting it transovarially from generation to generation (30). The identity of the Neomelaniconion species reported as lineatopennis in all of these studies is in doubt; in Kenya the species if probably the recently described mcintoshi Huang (3), but in South Africa it may be an undescribed sibling species in this complex. The fact that Rift Valley fever virus has been isolated from several species of Neomelaniconion and is known to be transmitted horizontally or vertically by some of these mosquitoes underscores the importance of obtaining basic information on the systematics and biology of species of Neomelaniconion, for such information is critical to a complete understanding of the natural history of Rift Valley fever virus.

Approach to the Problem

A modern systematic study of Neomelaniconion, utilizing morphological characteristics from both sexes and all stages in the life cycle, will be undertaken in order to determine the number of species in the subgenus, the most reliable means of distinguishing these species from each other, the existence and nature of intraspecific variation, the geographic distribution of the species, and the evolutionary relationships of the species. The results of this study will be published in a monograph that will include: taxonomic

descriptions of species and groups of species; identification keys for all stages in the life cycle; detailed drawings of the larva, pupa, and male genitalia of each species and of the adult morphology for selected species; photographs of eggs; information on type specimens; synonymies; discussions of diagnostic characters, variation, and relationships; summaries of bionomics and medical importance; data on geographical distribution of the species, including lists of specimens examined and maps; and a bibliography.

Although the historically important specimens of Neomelaniconion currently held in museums will be examined, the bulk of the specimens studied will be collected specifically for the project. The collection, rearing, and preservation of material and the recording of field data will follow the procedures developed for the "Mosquitos of Middle America" project (31). Emphasis will be placed on collecting adult females, from which eggs for progeny rearings and laboratory experiments can be obtained, and on collecting the immature stages, which can be reared individually. Both progeny rearings and individual rearings associate the stages and sexes of a species unequivocally. Specimens collected in the field or borrowed from museums will be prepared for study using standard laboratory procedures for mosquitoes, in general following the methods of Belkin (19). Classical, comparative morphological taxonomic procedures will be emphasized, as outlined for mosquito systematics by Belkin (19) and Zavortink (32). The form of presentation and terminology used in the final monograph will follow Belkin (19) and Zavortink (33-35) in large part.

The initial phases of research on the "Biosystematics of Aedes (Neomelaniconion)" project must, out of necessity, emphasize training of staff, development of field and laboratory techniques, field work to collect and rear specimens, and laboratory work to prepare the specimens for critical study.

Results and Discussion

Accomplishments related to the goal of producing a monograph of the subgenus Neomelaniconion that were completed during the second contract year of the project "Biosystematics of Aedes (Neomelaniconion)" are described below.

FACILITIES

In addition to the laboratories obtained during the first contract year, one additional laboratory of approximately 175

square feet has been acquired for the project. This room is adjacent to the laboratory used for rearing mosquitoes, and is also used to rear specimens.

A second insect storage cabinet with 25 Cornell drawers was purchased. This has provided much needed room for expansion and reorganization of the *Aedes (Neomelaniconion)* collection. Additional memory was obtained for the IBM PC/XT purchased for the project. The added memory makes it possible to run software for data manipulation and for phylogenetic analysis of systematic data.

STAFF

The following full-time and part-time staff were supported by the contract during the second year:
Thomas J. Zavortink, Principal Investigator (50% time)
Sandra S. Shanks, Taxonomic Research Specialist (100% time)
Mary Ann Tenorio, Scientific Illustrator (Piecework)

COOPERATORS

The following individuals contributed to the "Biosystematics of *Aedes (Neomelaniconion)*" project during the second contract year:

Maureen Coetzee, South African Institute for Medical Research, Johannesburg, organized the Principal Investigator's field trip to South Africa, helped with the collection and rearing of *Neomelaniconion*, and provided lodging in her home.

Roger Cordellier, Chief, ORSTOM Medical Entomology Laboratory, Abidjan, Ivory Coast, arranged for transportation, lodging, laboratory facilities, and field help during the Principal Investigator's trip to Ivory Coast, and provided access to ORSTOM field stations.

Anton Cornel, South African Institute for Medical Research, accompanied the Principal Investigator in the field in South Africa, and helped with the collection and rearing of *Neomelaniconion*.

A. Ehouman, Director, Institute Pasteur, Ivory Coast, authorized provision of laboratory facilities, vehicles, chauffeurs, and field collectors during the trip to Ivory Coast.

Bernard Geoffroy, ORSTOM, Montpellier, France, made all the contacts for the trip to Ivory Coast, conducted joint field research with the Principal Investigator in Ivory Coast, and helped with the collection of *Neomelaniconion*.

Richard H. Hunt, Head, Department of Entomology, South African Institute for Medical Research, arranged for transportation, lodging, and laboratory facilities during the trip to South Africa, helped with the collecting and rearing

of *Neomelaniconion*, and gave permission to study the Institute's collection of *Neomelaniconion*.

Peter Jupp, National Institute for Virology, Johannesburg, South Africa, provided information on collecting sites in South Africa, and gave permission to study the Institute's collection of *Neomelaniconion*.

A. Kouasai, Minister of Scientific Research, Ivory Coast, provided authorization to conduct research in that country.

Bruce McIntosh, Port Shepstone, South Africa, provided valuable information on collecting sites for South African *Neomelaniconion*, provided lodging and laboratory facilities at his home, and helped with the collecting of *Neomelaniconion*.

Rudy Meiswinkle, Veterinary Research Institute, Onderstepoort, South Africa, provided access to a collecting area at one of the Institute's farms and provided lodging in his home.

J. Metz, Director, South African Institute for Medical Research, authorized provision of laboratory facilities, vehicles, lodging, and field assistance during the trip to South Africa.

Bernard Mondet, ORSTOM, Abidjan, facilitated the collection of *Neomelaniconion* in Ivory Coast.

Brian Sharp, Research Institute for Diseases in a Tropical Environment, Durban, South Africa, provided information on collecting areas in the coastal portion of Natal and provided lodging at the Institute's house in Jozini.

Sangare Yaya, Institute of Tropical Ecology, gave permission to conduct field research and utilize the facilities at the Institute's field station at Tai, Ivory Coast.

ACQUISITION OF SPECIMENS

Loans from Museums. - Small loans of *Neomelaniconion* specimens were received from the Institute Pasteur, Paris; the National Institute for Virology, Johannesburg; and the South African Institute for Medical Research, Johannesburg. A larger loan of *Neomelaniconion* males and male genitalia from Central African Republic was received from Bernard Geoffroy, ORSTOM, Montpellier, France. This loan is of particular interest because it documents the existence of 11 species of *Neomelaniconion*, including two undescribed ones, in the Central African Republic.

Collecting and Rearing. - Mosquitoes were collected and reared in Ivory Coast during June and July 1987 (112 collections) and in South Africa during February, March, and April 1988 (57 collections). Eleven species of *Neomelaniconion*, including four or five undescribed ones, were collected in Ivory Coast. Five of the six species of

Neomelaniconion known to occur in South Africa were collected during the trip to that country. In addition, one of the three populations of *Aedes mcintoshi* collected there differs slightly from the others, and it may represent an undescribed sibling species.

During the field trips to Ivory Coast and South Africa, the collection of adult female *Neomelaniconion* was emphasized. The females were isolated in vials, given a blood meal, and many lived to lay one or more clutches of eggs. These clutches of eggs were brought to the University of San Francisco, and progeny series of specimens were reared from them in the laboratory. Two-hundred and six clutches of *Neomelaniconion* eggs were obtained in Ivory Coast, and 176 were obtained in South Africa.

There are several advantages to collecting adult female *Neomelaniconion* in the field, obtaining eggs from them, and rearing progeny in the laboratory. These are: 1) the time interval during which females can be collected is much longer than that during which larvae can be found because females survive for several weeks in the vicinity of the pools from which they have emerged, whereas larvae can be collected for only a few days after the initial flooding of their habitat, 2) eggs are available for taxonomic study, 3) all specimens are reared under the same conditions, thus assuring that morphological differences observed between populations have a genetic basis, 4) the sexes and stages of each species are unquestionably associated, 5) the range of variation in the progeny of one female can be documented, and 6) live material is available in the laboratory and can be used for experimentation or frozen to provide electrophoretic data at a later time.

Great difficulty has been experienced in rearing larvae of several *Neomelaniconion* species from Ivory Coast and South Africa. The standard procedure for hatching eggs and rearing larvae developed during the first contract year works well for species that breed in grassy, sunlit pools, particularly if large numbers of larvae hatch from an egg clutch. However, this procedure is not suitable for those species that breed in other habitats, such as deeply shaded forest pools, and it is not suitable for any species when only a few eggs hatch and only a few larvae are present. Much experimenting has been done, and is still being done, in an attempt to develop better rearing procedures. Different hatching methods, different water temperatures, pH's, and hardnesses, and different foods have been tried. Discussion of the difficulty of rearing *Neomelaniconion* larvae with George B. Craig, Jr., and observation of the rearing techniques used at the Vector Biology Laboratory during a visit to the University of Notre Dame in January 1988 have

provided new insights. The Principal Investigator's current hypothesis is that larvae of many Neomelaniconion species are easily overwhelmed by bacteria, and it is therefore a mistake to use nutrient broth to promote the growth of bacteria as a means of lowering the oxygen content of the rearing medium in order to stimulate egg hatching. Experimentation is currently centering on using physical means of removing oxygen from the water in order to stimulate egg hatching, and on finding an alternate food for newly hatched larvae.

In the absence of a well-defined hatching and rearing procedure that works for all species, several of the more delicate species have been reared with limited success in aged tap water to which a little soil or dried leaves of grass, big-leaf maple (*Acer macrophyllum*), or privet (*Ligustrum lucidum*) have been added.

PREPARATION OF SPECIMENS FOR STUDY

All mosquitoes collected in Ivory Coast or reared at the University of San Francisco from eggs obtained in Ivory Coast have been prepared for study. Specimens collected and reared in South Africa are currently being prepared for study, and egg clutches obtained in South Africa are currently being flooded and the larvae reared. Approximately 3800 adult mosquitoes were mounted on points and approximately 1500 microscope slides of immature mosquitoes (whole larvae and larval and pupal exuviae associated with adults) were prepared during the second contract year. About 75% of the specimens from Ivory Coast were provided with printed locality labels. One hundred microscope slides of male genitalia were prepared during the year.

By the end of the second contract year, a total of 7313 pinned adults and 3965 microscope slides (3802 of immature mosquitoes, 163 of male genitalia) had been prepared on the project.

IDENTIFICATION

Specimens of Neomelaniconion borrowed during the second contract year or examined at the National Institute for Virology and the South African Institute for Medical Research during the Principal Investigator's stay in Johannesburg were identified. Twenty species were represented in this material. These species and the countries in which they were collected are:

Aedes (Neomelaniconion)
- *albicosta* (Edwards) Ethiopia
- *albothorax* (Theobald) Mozambique
- *aurovenatus* Worth South Africa

bequaerti Wolfs Zaire
bergerardi Pajot & Geoffroy Central African Republic
bolensis Edwards Central African Republic
carteri Edwards Central African Republic
circumluteolus (Theobald) Central African Republic, Mozambique, South Africa, Zaire
crassiforceps Edwards Central African Republic
jamoti Hamon & Rickenbach Central African Republic
lineatopennis (Ludlow) Philippines
luridus McIntosh South Africa
luteolateralis (Theobald) South Africa
mcintoshi Huang Bechuanaland, South Africa, Zambia, Zimbabwe
palpalis (Newstead) Central African Republic,
pogonurus Edwards Central African Republic
taeniarostris (Theobald) Central African Republic
unidentatus McIntosh Basutoland, South Africa
undescribed species #1 (affinities unknown) Central African Republic
undescribed species #5 (*palpalis* group) Central African Republic

All specimens from the United States National Museum and British Museum of Natural History determined as *Aedes (Neomelaniconion) fuscinervis* (Edwards) during the first contract year were examined again and redetermined as follows:

Aedes (Neomelaniconion)
fuscinervis (Edwards) Ghana, Nigeria
undescribed species #6 (*fuscinervis* group?) Gambia, Liberia

During the second contract year, 16 species of *Neomelaniconion* were collected and reared for the project. These species and their geographic origins are:

Aedes (Neomelaniconion)
carteri Edwards Ivory Coast
circumluteolus (Theobald) Ivory Coast, South Africa
fuscinervis (Edwards) Ivory Coast
jamoti Hamon & Rickenbach Ivory Coast
luridus McIntosh South Africa
luteolateralis (Theobald) South Africa
?maculicosta Edwards Ivory Coast
mcintoshi Huang South Africa
punctocostalis (Theobald) Ivory Coast
taeniarostris (Theobald) Ivory Coast
unidentatus McIntosh South Africa

undescribed species #1 (affinities unknown) Ivory Coast
undescribed species #2 (_fuscinervis_ group?) Ivory Coast
undescribed species #3 (_fuscinervis_ group?) Ivory Coast
undescribed species #4 (affinities unknown) Ivory Coast
undescribed species #7 (near _mcintoshi_) South Africa

During the second contract year, very little time was devoted to identifying mosquitoes other than _Neomelaniconion_ collected in Africa by the project's staff. None of the backlog of unidentified specimens from Kenya and Zambia was determined, and only some of the species collected in Ivory Coast and South Africa were provisionally identified on the basis of external adult characters, as follows:

Aedes (Aedimorphus)
- _bevisi_ (Edwards) South Africa
- _cumminsii_ (Theobald) South Africa
- _dalzieli_ (Theobald) Ivory Coast
- _dentatus_ (Theobald) South Africa
- _domesticus_ (Theobald) group Ivory Coast
- _?fowleri_ (Charmoy) Ivory Coast
- _hirsutus_ (Theobald) South Africa
- _microstictus_ Edwards South Africa
- _minutus_ (Theobald) Ivory Coast
- _veeniae_ McIntosh South Africa

Aedes (Albuginosus)
- _marshallii_ (Theobald) South Africa
- _stokesi_ (Evans) Ivory Coast

Aedes (Finlaya)
- _fulgens_ (Edwards) South Africa
- _longipalpis_ (Gruenberg) Ivory Coast

Aedes (Mucidus)
- _grahamii_ (Theobald) Ivory Coast
- _mucidus_ (Karsch) Ivory Coast

Aedes (Ochlerotatus)
- _caballus_ (Theobald) South Africa
- _juppi_ McIntosh South Africa

Aedes (Pseudarmigeres)
- _kummi_ Edwards Ivory Coast

Aedes (Stegomyia)
- _demeilloni_ Edwards South Africa
- _ledgeri_ Huang South Africa
- _lilii_ (Theobald) Ivory Coast
- _metallicus_ (Edwards) South Africa
- _simpsoni_ (Theobald) South Africa
- _strelitziae_ Muspratt South Africa

Coquillettidia (Coquillettidia)
microannulata (Theobald) South Africa
Culex (Culex)
theileri Theobald South Africa
univittatus Theobald group South Africa
Culex (Eumelanomyia)
albiventris Edwards Ivory Coast
horridus Edwards South Africa
Culex (Lutzia)
tigripes De Grandpre & De Charmoy Ivory Coast
Culiseta (Allotheobaldia)
longiareolata (Macquart) South Africa
Eretmapodites
chrysogaster Graham group Ivory Coast
?dracaenae Edwards Ivory Coast
?grahami Edwards Ivory Coast
leucopus Graham group Ivory Coast
oidipodeios Graham group Ivory Coast
quinquevittatus Theobald South Africa
silvestris Ingram & DeMeillon South Africa
Toxorhynchites (Toxorhynchites)
brevipalpus ssp. conradti Gruenberg Ivory Coast
erythrurus (Edwards) Ivory Coast
phytophagus Theobald Ivory Coast
Uranotaenia (Pseudoficalbia)
ornata Theobald Ivory Coast

ILLUSTRATION

During the second contract year preliminary pencil drawings were completed for the larvae and male genitalia of eight species of Neomelaniconion, and the pupae of nine species. These drawings must be checked and corrected for the modal number of branches in each seta in a sample of 10 specimens before they can be inked.

Scanning electron micrographs of the eggs of 14 species of Neomelaniconion were prepared during the year.

TAXONOMIC STUDY

The study of type specimens is essential in monographic studies, and this aspect of the research on Neomelaniconion was started during the second contract year. Holotypes of Aedes aurovenatus, Aedes luridus, and Aedes unidentatus were examined at the South African Institute for Medical Research and the National Institute for Virology in Johannesburg, South Africa.

The preparation of a bibliography for the subgenus _Neomelaniconion_ was continued, and many additional important papers on _Neomelaniconion_ and other African mosquitoes were obtained for the project's files.

Specimens collected and reared for the project during the second contract year have contributed significantly to an understanding of the subgenus _Neomelaniconion_. Eleven species of _Neomelaniconion_ were collected in Ivory Coast. Egg clutches of nine of these species were brought back to San Francisco and reared. All stages - eggs, larvae, pupae, and adults - of these nine species are now known, and the sexes are definitely associated. These species are _carteri_, _circumluteolus_, _fuscinervis_, _jamoti_, _?maculicosta_, _punctocostalis_, _taeniarostris_, and undescribed species 1 and 2. The other two species collected in Ivory Coast, undescribed species 3 and 4, are represented by field collected males only. Progeny rearings have shown that _monotrichus_ is the male of _punctocostalis_, so the former name must be relegated to synonymy. _Aedes maculicosta_ is currently considered to be a synonym of _carteri_ (1), but this can not be the case, and it is either a valid species or a synonym of _punctocostalis_. The Ivory Coast species provisionally identified as _maculicosta_ is very different from both _carteri_ and _punctocostalis_ in larval and male genitalic characters. If the type of _maculicosta_ is conspecific with _punctocostalis_, which seems likely on the basis of published descriptions, then the Ivory Coast species provisionally identified as _maculicosta_ is another undescribed species. Undescribed species number 1 is remarkable in several ways. The larva lives among fallen leaves in the bottom of forest pools and seldom comes to the water surface; it has very long anal gills with large, darkly pigmented tracheae, similar to those of the North American species _Aedes (Ochlerotatus) dupreei_ (Coquillett). Some features of the larva suggest that it is neotenic relative to other species of _Neomelaniconion_. The adult female displays incredible variation in wing markings; some individuals have large yellow spots on the anterior part of the wing, others have small white spots, and yet others have no spots at all. The genitalia of this species are similar to those of the savanna group of species, as defined by Edwards (5). Undescribed species 2 and 3 share some genitalic features with _fuscinervis_, and are tentatively included in a group with that species. The Ivory Coast species of _Neomelaniconion_ are, on the whole, well-differentiated from each other in larval and male genitalic characters; adult females are so variable, though, that determination of the species in this sex is sometimes very difficult.

After discovery of two undescribed species in the fuscinervis group in Ivory Coast, specimens from Gambia and Liberia questionably determined as fuscinervis during the first contract year were reexamined and found to represent yet another undescribed species (number 6) in this group.

At least five, and possibly six, species of Neomelaniconion were collected in South Africa and egg clutches of all of these were brought back to San Francisco where they are currently being reared. These species are circumluteolus, luridus, luteolateralis, mcintoshi, unidentatus, and a possible new species very similar to mcintoshi (?undescribed species number 7). Three populations of mcintoshi, as it is currently interpreted, were collected in South Africa: the topotypic one from Onderstepoort, one from the south coast of Natal Province, and one from the highveld in northern Orange Free State. This latter population displays subtle differences from the other two in egg shape, adult coloration, and development of setae of the male genitalia. It may represent a new species. Larvae and pupae of these three South African populations of mcintoshi are unstudied at this time because they have not been slide-mounted yet. A final decision on the status of the highveld population must await examination of the immature stages and possibly even the availability of electrophoretic data. In contrast to the Ivory Coast species of Neomelaniconion, the South African species of the subgenus are scarcely differentiated from each other in larval or male genitalic characters; they are, instead, best separated from each other by slight differences in coloration of the females.

Examination of Neomelaniconion borrowed from other collections during the second contract year has also contributed greatly to understanding this subgenus. While borrowed specimens are often not of high enough quality for complete description or illustration, they can be extremely valuable in checking diagnostic characters and in determining geographic ranges of the species. The most important loan of specimens studied during the year is that of males and male genitalia of Neomelaniconion collected in Central African Republic by Bernard Geoffroy. Eleven species are included in this loan. Two of these, bergerardi and undescribed species number 5, are known only from Central African Republic. For six of the other species, the Central African Republic records are either the southern-most, northern-most, or eastern-most known for these species. Specimens in this loan have contributed also to an understanding of the palpalis complex. The male genitalia of palpalis from Central African Republic show constant differences from the male genitalia of carteri from Ivory Coast, thus confirming the specific status of these taxa. Undescribed species number 5 also belongs to

the *palpalis* complex of species.

Study of the eggs of *Neomelaniconion* by means of scanning electron microscopy has shown the existence of numerous previously unknown taxonomic characters of specific and group value. Eggs of *Neomelaniconion* species may differ in size, shape, and extent and nature of chorionic sculpturing. Egg characters seem to confirm Edwards's (5) division of the subgenus into two groups on the basis of abdominal markings of the adult female. The eggs of several species in Edwards's savanna group are relatively short and broad, and lack any conspicuous chorionic sculpturing. The eggs of several species in his forest group are long and slender, and have conspicuous chorionic sculpturing at both ends. The egg of only one species of *Neomelaniconion*, undescribed species number 2, has the entire surface of its chorion sculptured.

An electrophoretic study of enzymes of *Aedes (Neomelaniconion)* was initiated in cooperation with Leonard Munstermann of the Vector Biology Laboratory, University of Notre Dame. In mosquito systematics, electrophoretic data are used primarily for species discrimination (36-38), but they can be analyzed by phenetic or phylogenetic methods to show relationships of species (39-41). Preliminary research done at the University of Notre Dame in January 1988 showed that *Neomelaniconion* is very amenable to enzyme analysis. Of particular interest was the discovery that the morphologically similar species that are widespread in East Africa, namely *circumluteolus*, *mcintoshi*, and *unidentatus*, differ more from each other in their isozymes than do the morphologically distinct species of West Africa included in the analysis. The Principal Investigator believes that study of enzyme variation in *Neomelaniconion* would be of the greatest utility in detecting morphologically cryptic species, particularly in the savanna group, and it is hoped that it will be possible to continue and expand the electrophoretic study started this year.

Conclusions

The following are concluded as a result of the second year's activities of the project:

1. Numerous undescribed species of *Neomelaniconion* exist. Most of these occur in the western and central parts of Africa, but even the relatively well-studied *Neomelaniconion* fauna of South Africa may include a morphologically cryptic species.

2. Changes in the taxonomy of the known species of Neomelaniconion must be made. Aedes monotrichus must be reduced to synonymy with punctocostalis. Aedes maculicosta is not a synonym of carteri, but either a distinct species or a synonym of punctocostalis.

3. The eggs of Neomelaniconion provide taxonomic characters of both specific and group significance. Egg characters seem to support the division of Neomelaniconion into two major groups, a forest group and a savanna group.

4. Enzyme analysis of Neomelaniconion shows great promise both for detecting morphologically cryptic species and for providing an alternate data base for determining the relationships of species.

5. The Ivory Coast species of Neomelaniconion, nearly all of which belong to the forest group of species, are, for the most part, well-differentiated from each other in larval and male genitalic characters. Because of the extreme variation in colorational characters shown by females of several species, identification of this sex is often very difficult. The species of the forest group from Ivory Coast for which electrophoretic data are available are remarkably similar in their isozymes.

6. The South African species of Neomelaniconion, all of which belong to the savanna group of species, have very similar larvae and male genitalia. The species are distinguished primarily by colorational features of the females. Preliminary electrophoretic data for three species of the savanna group show that each of these species has several diagnostic isozymes.

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